MARKED-UP COPY OF FIGURES

Sorting by pools

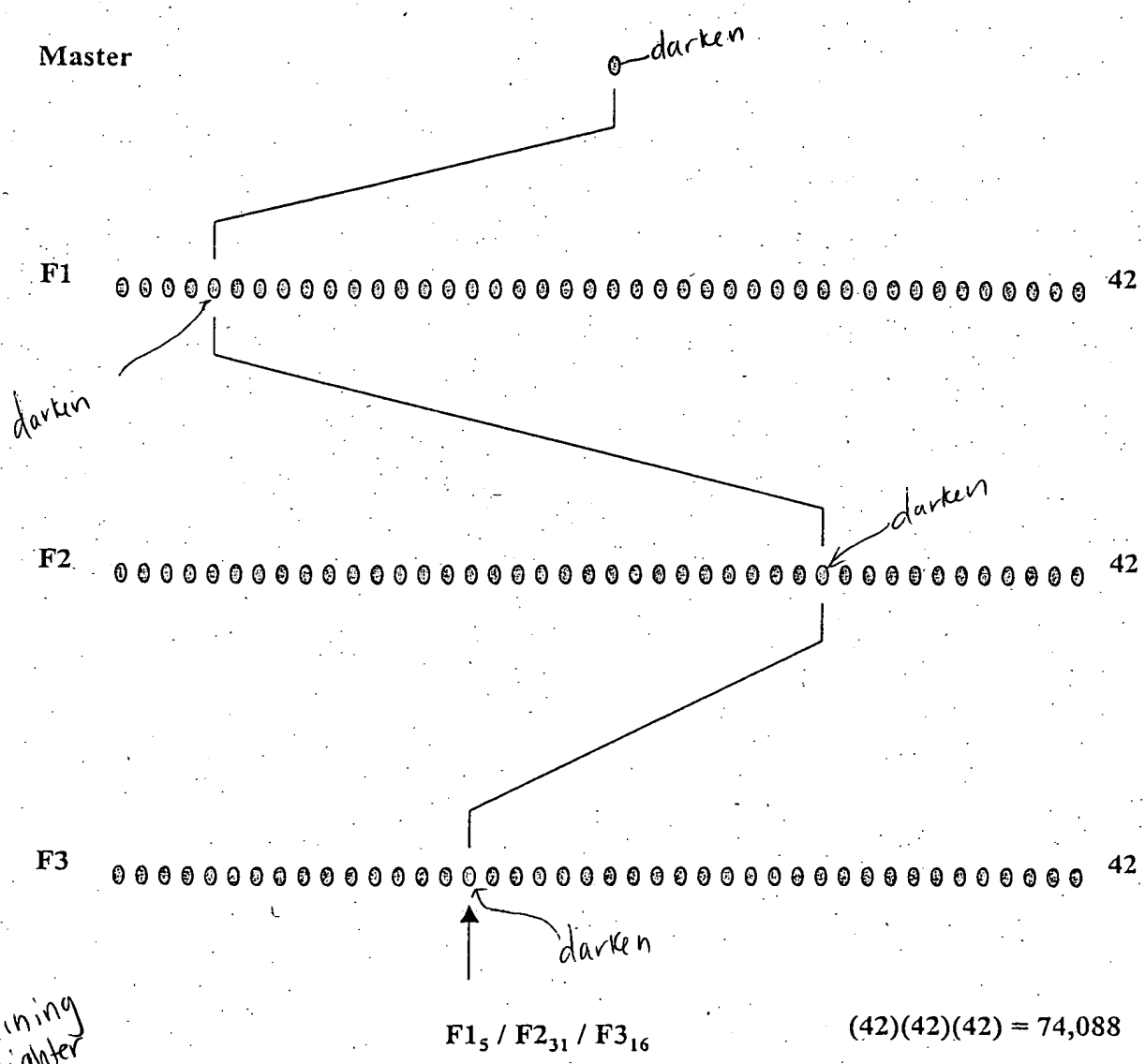


FIGURE 1

Sorting by pools: Decreasing pool diversities

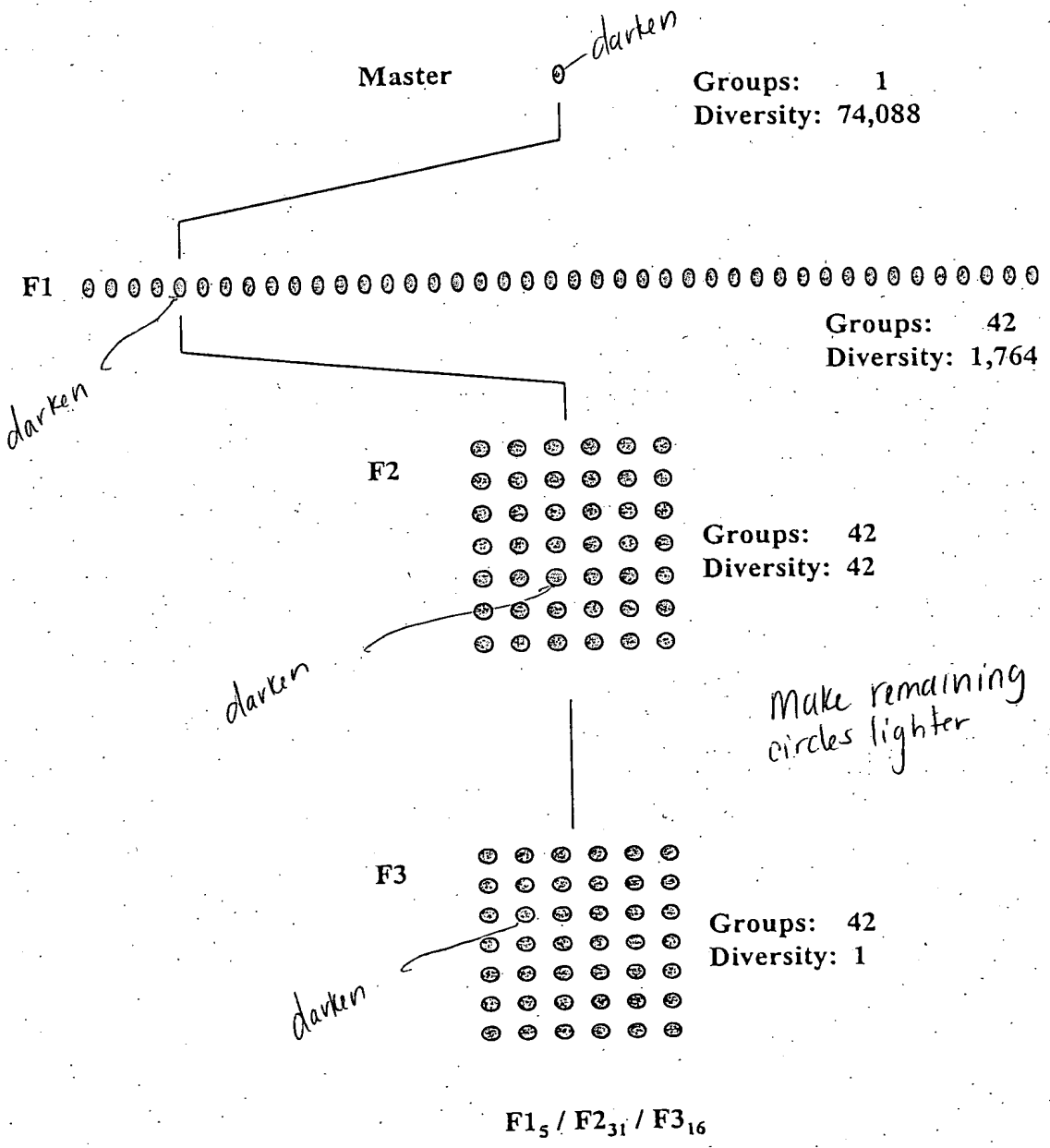
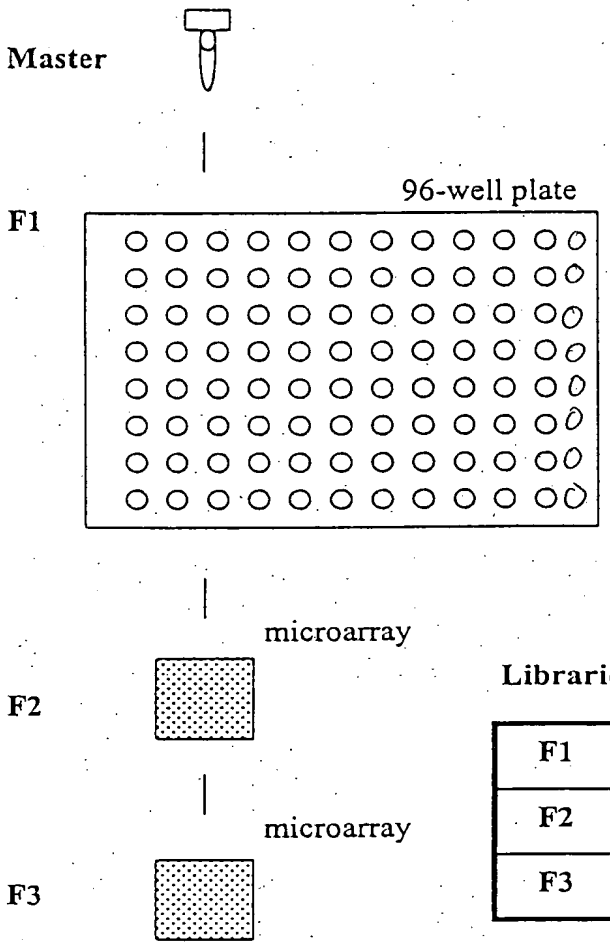


FIGURE 2

Sorting by pools: Screening large diversity libraries



Libraries	Pools			
F1	100	1,000	100	1,000
F2	100	100	1,000	1,000
F3	100	100	1,000	1,000
Totals	10^6	10^7	10^8	10^9

FIGURE 3

Searching a mutation library

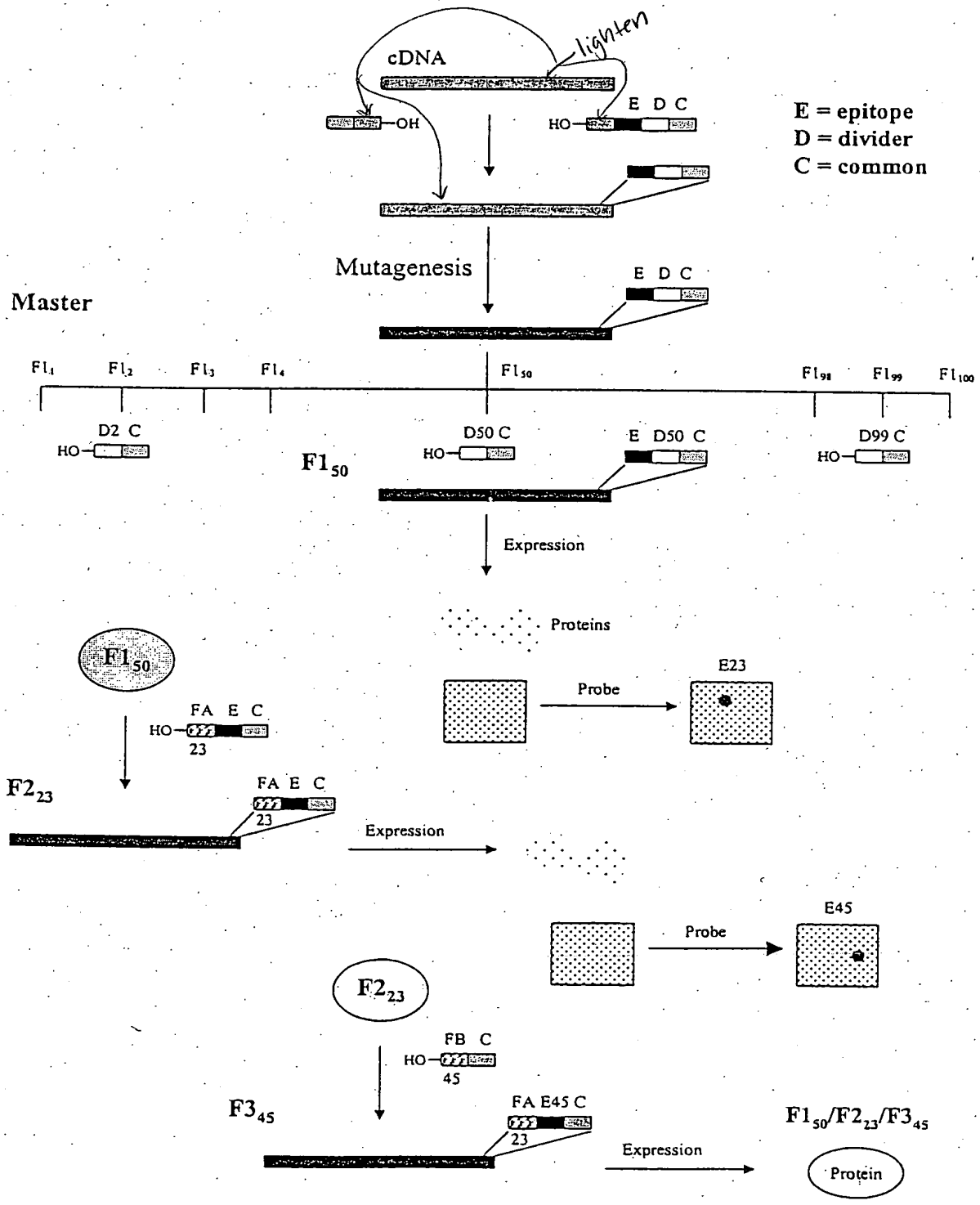


FIGURE 4

Making a recombinant antibody library

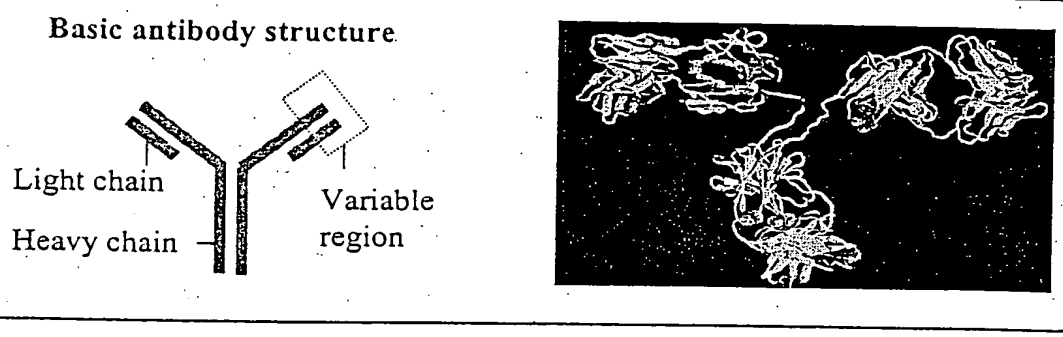


FIGURE 5

Searching a recombinant antibody library

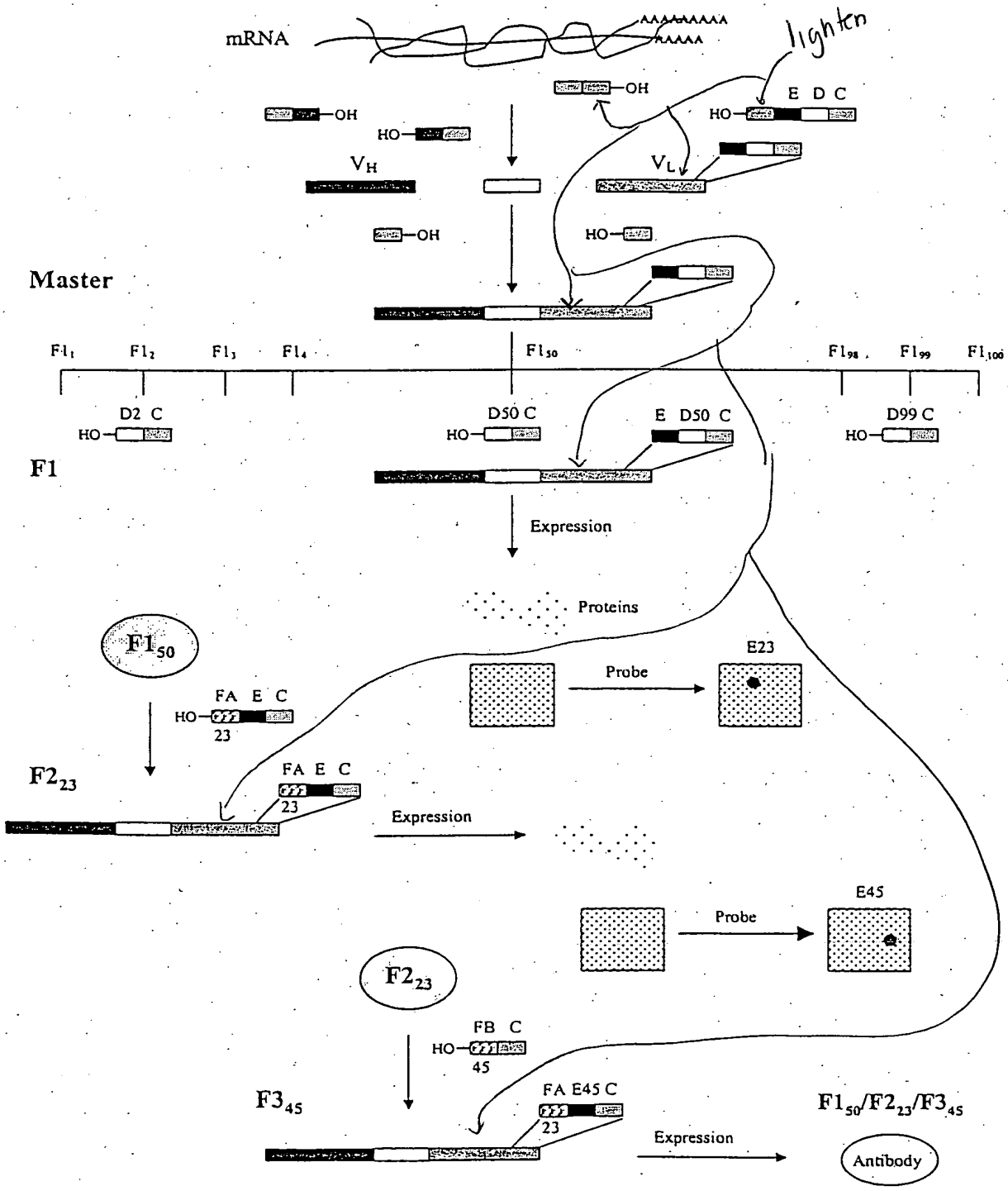
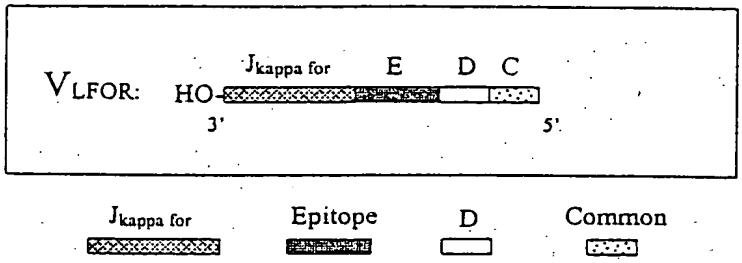
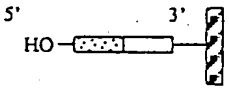


FIGURE 8

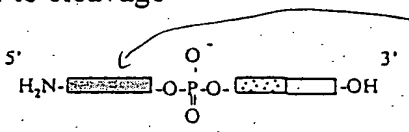
Making the V_{LFOR} primers: Solid phase synthesis



1. Synthesize oligo on solid support

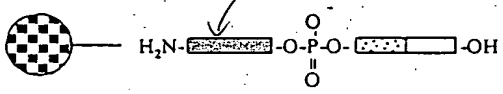


2. Add aminolink prior to cleavage



lighten

3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

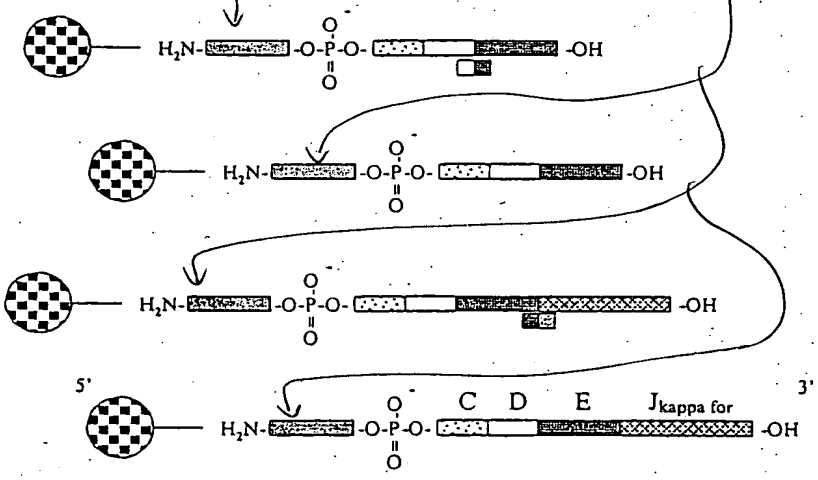


FIGURE 10

step II

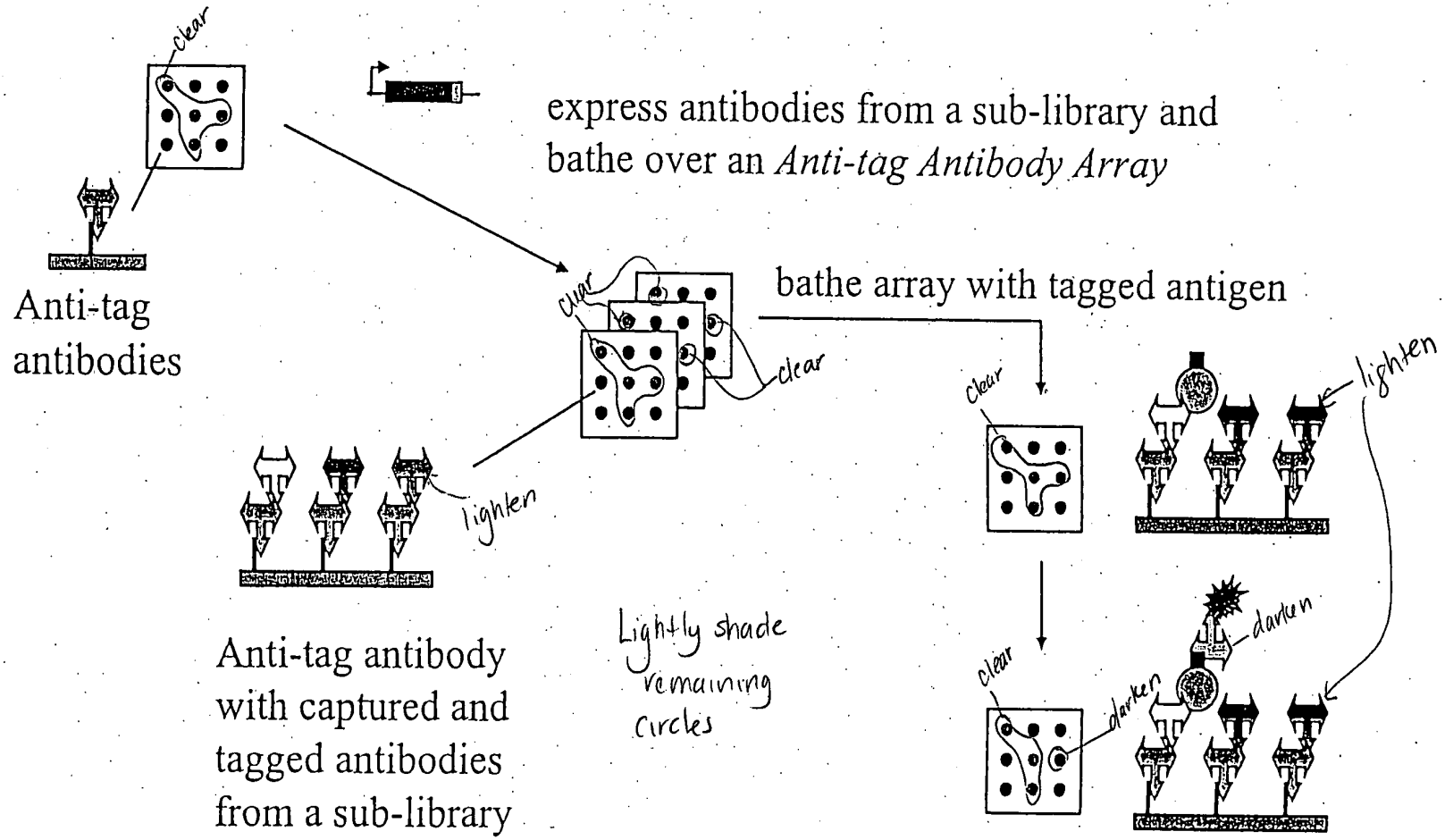
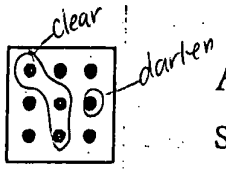


FIGURE 14B

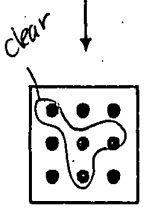
step III



Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

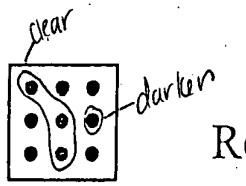
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

Express and purify the antibodies



Re-distribute over an *Anti-tag Antibody Array*

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb

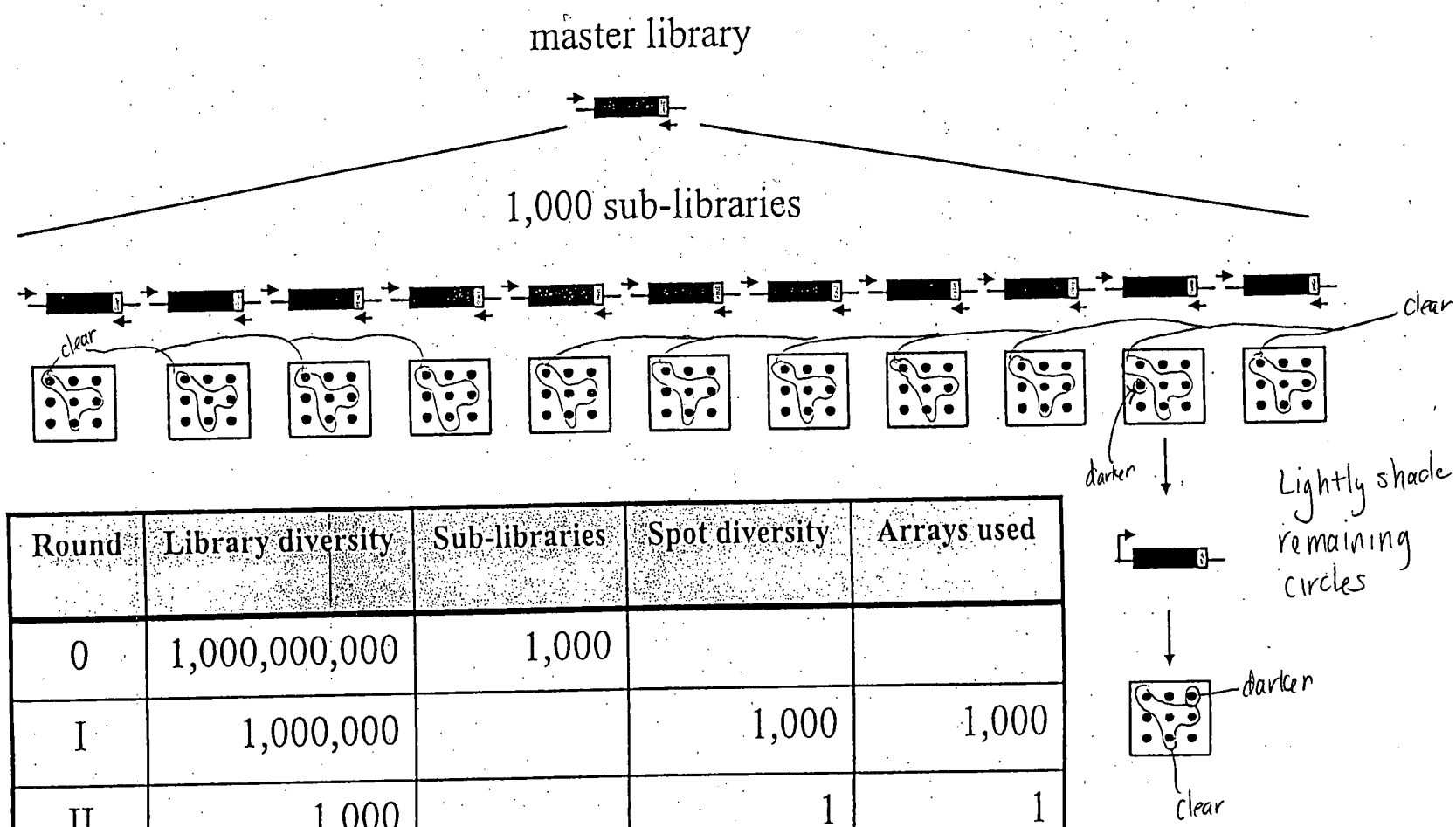


Re-survey to ID the antibody of interest

Lightly shade remaining circles

FIGURE 14C

summary



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIGURE 14D

- Modification searches

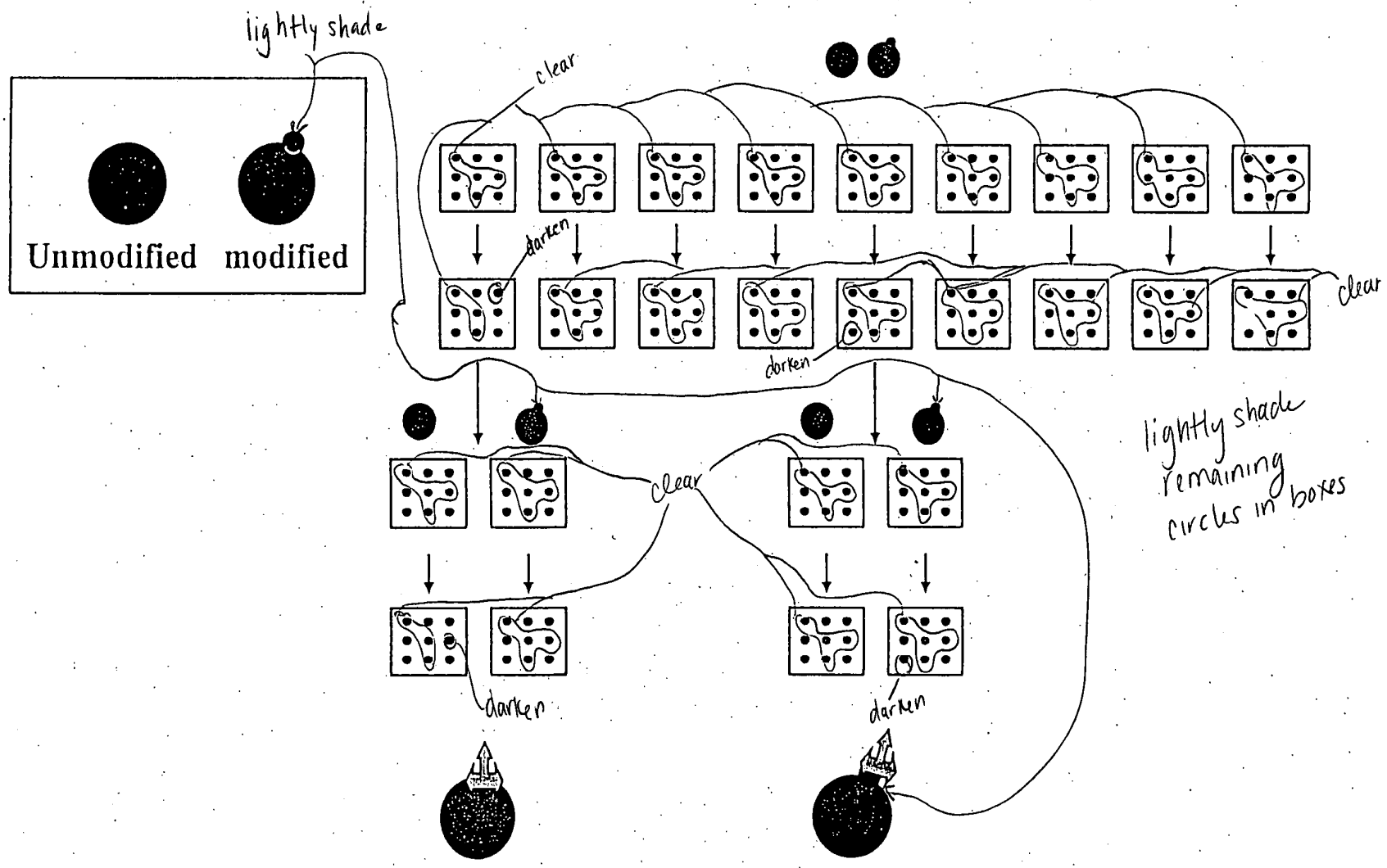


FIGURE 15

Simultaneous searches

Round Arrays Bait Probe

I 1,000 Abs Ags

II 1,000 Abs Ags

III 1,000 Ags Abs

3,000

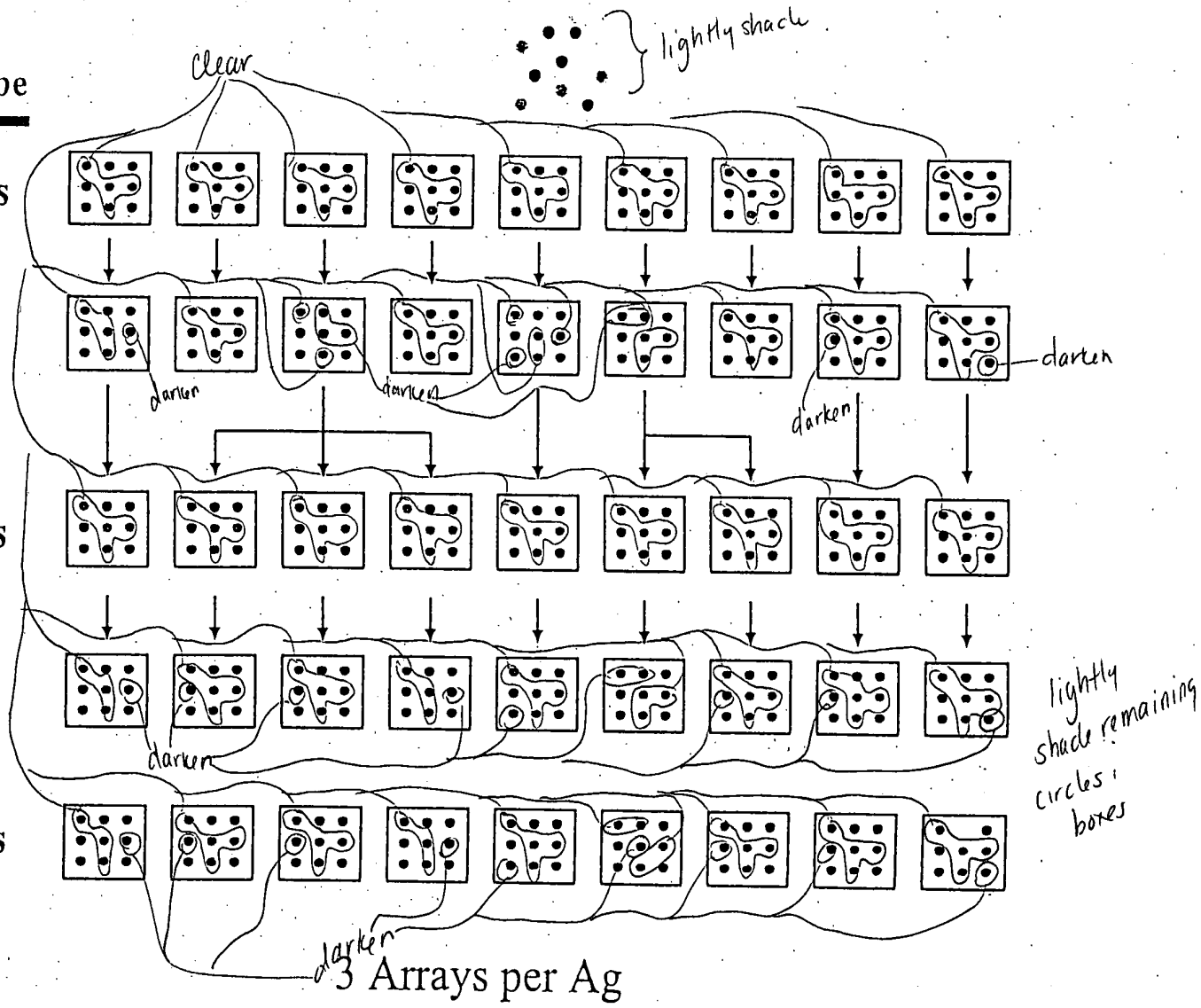
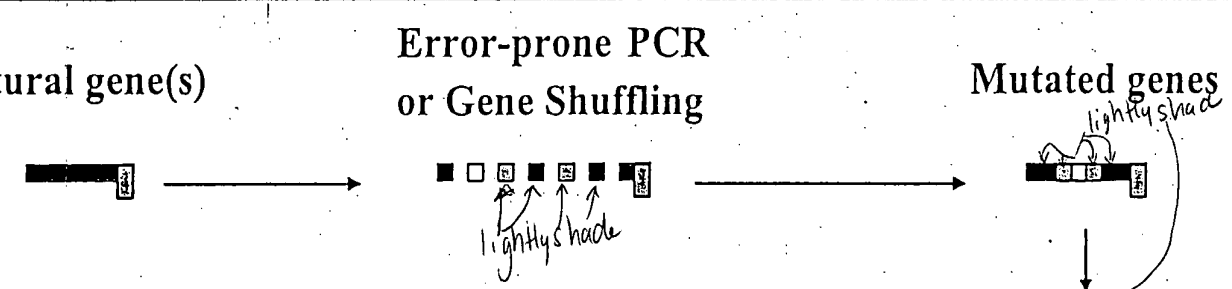
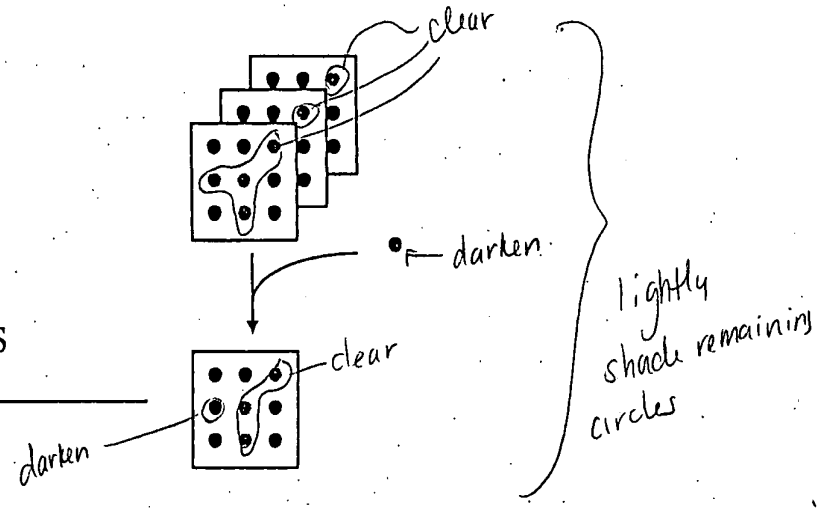


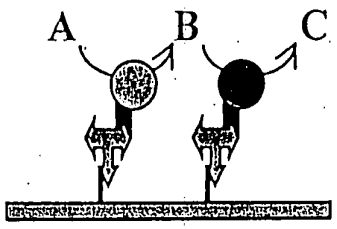
FIGURE 16

Enzyme engineering



- tag the gene to be mutated
- mutate genes and create sub-libraries
- distribute mutants over arrays
- probe the arrays with labeled substrates





Spots can contain mixtures of enzymes for detection or pathway engineering

FIGURE 17

Protein interaction mapping

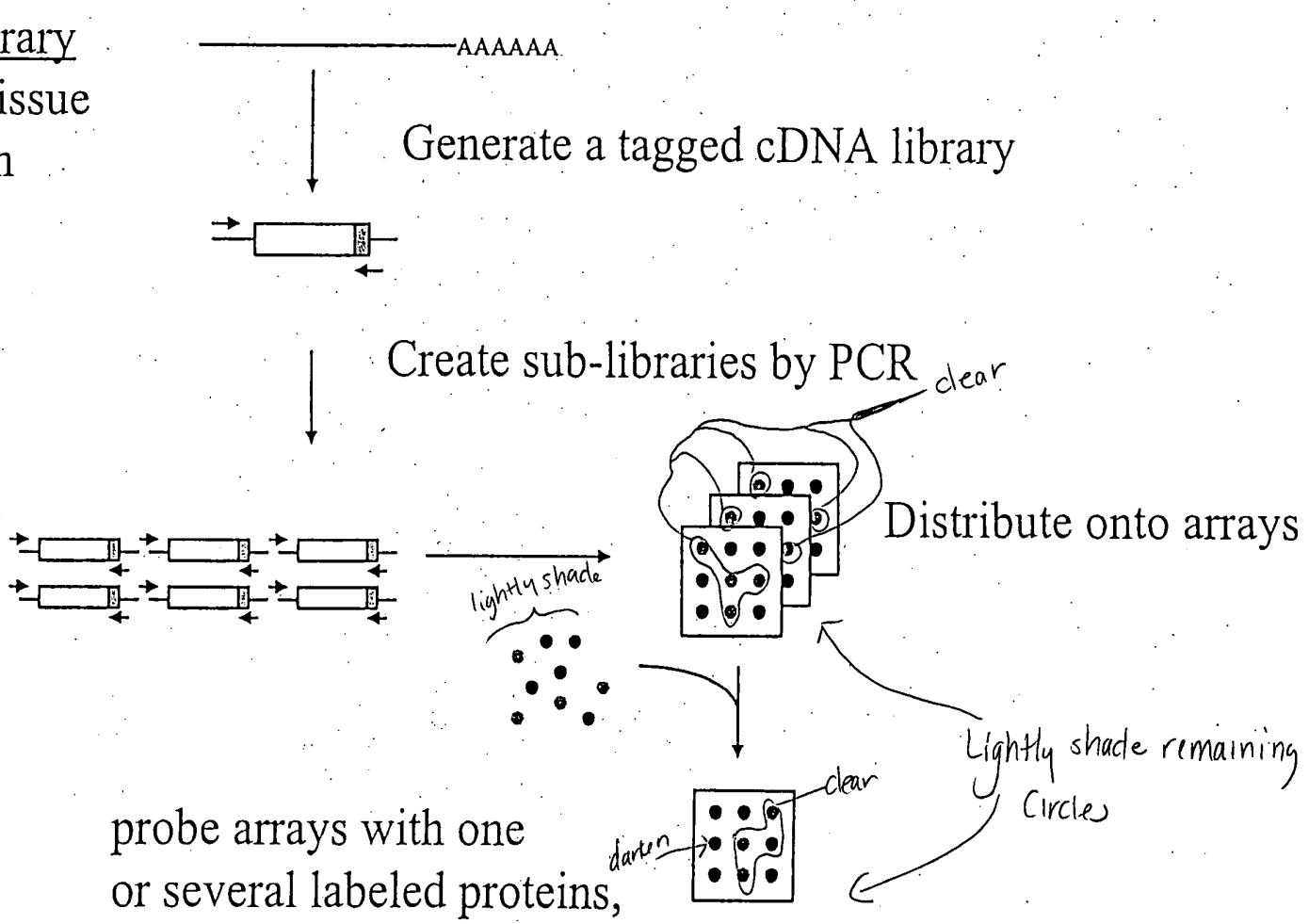


FIGURE 18

Sorting by pools

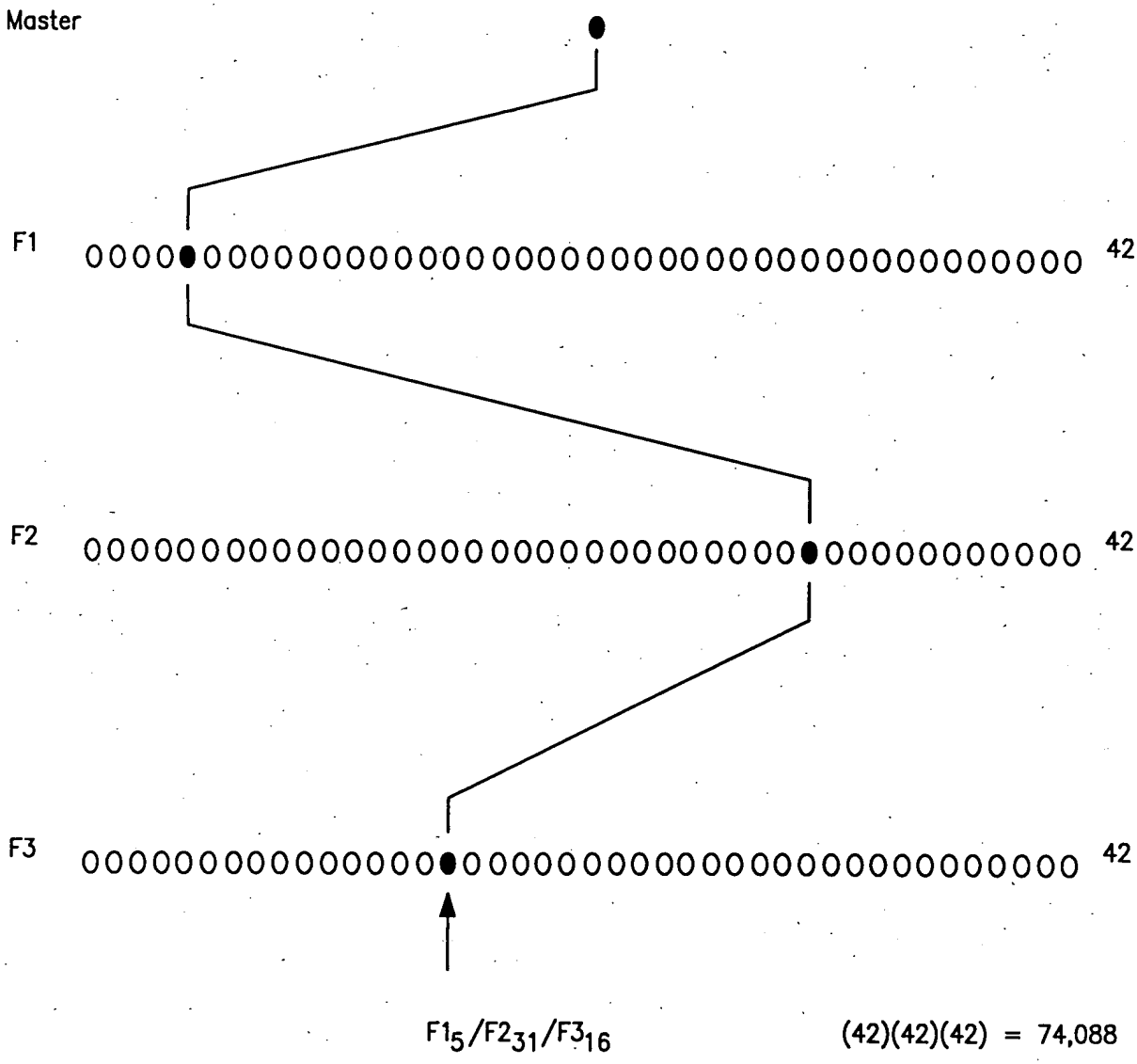


FIG. 1

Sorting by pools: Decreasing pool diversities

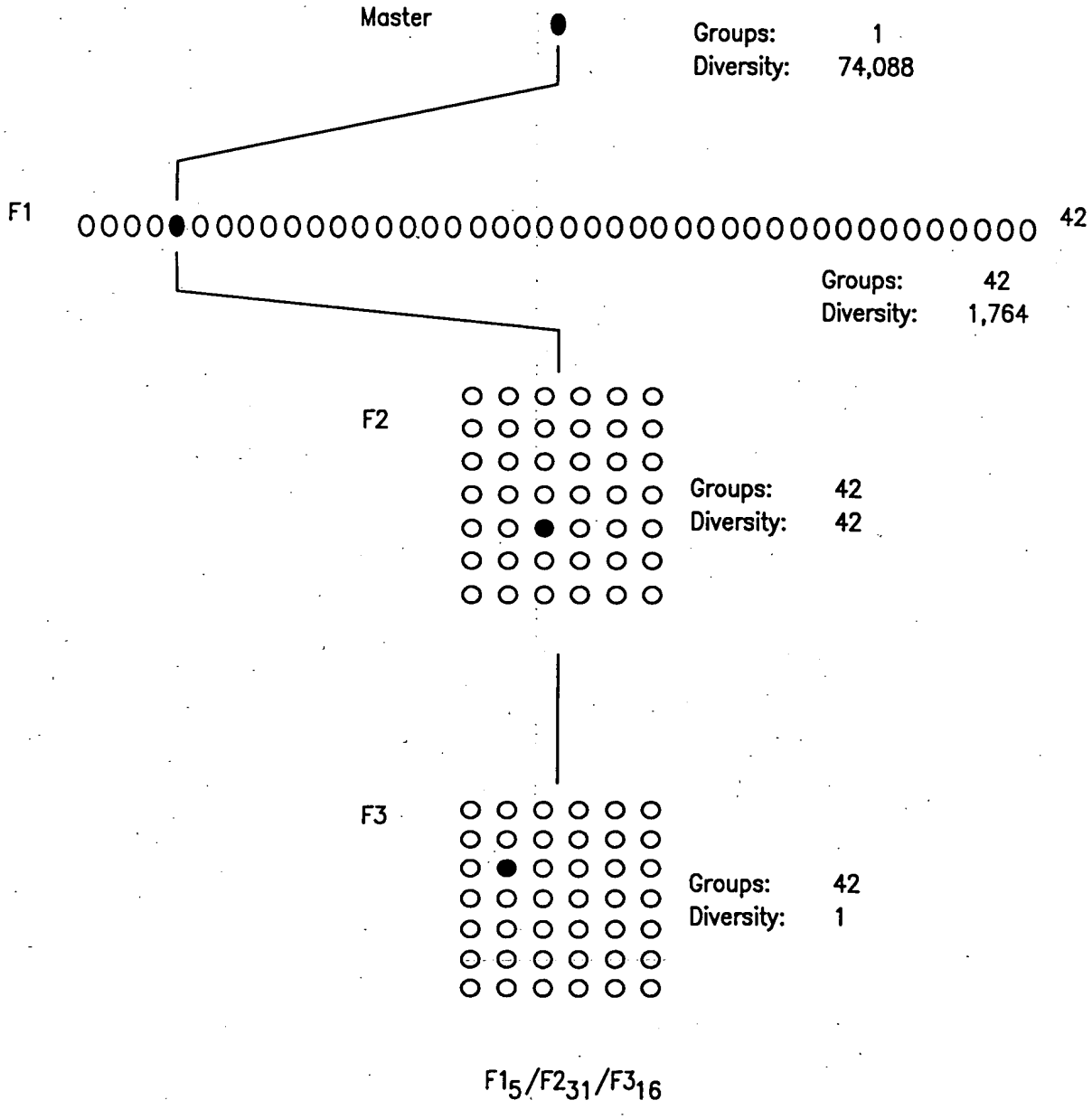
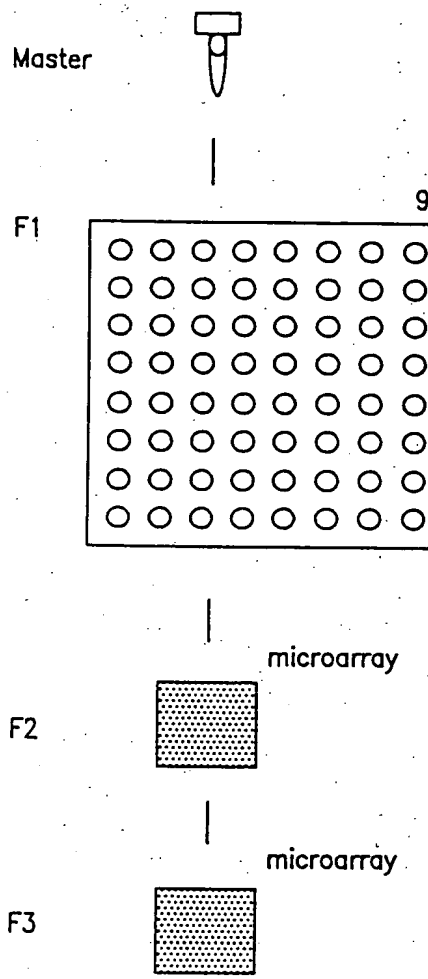


FIG. 2

Sorting by pools: Screening large diversity libraries



Libraries	Pools			
F1	100	1,000	100	1,000
F2	100	100	1,000	1,000
F3	100	100	1,000	1,000
Totals	10^6	10^7	10^8	10^9

FIG. 3

Searching a mutation library

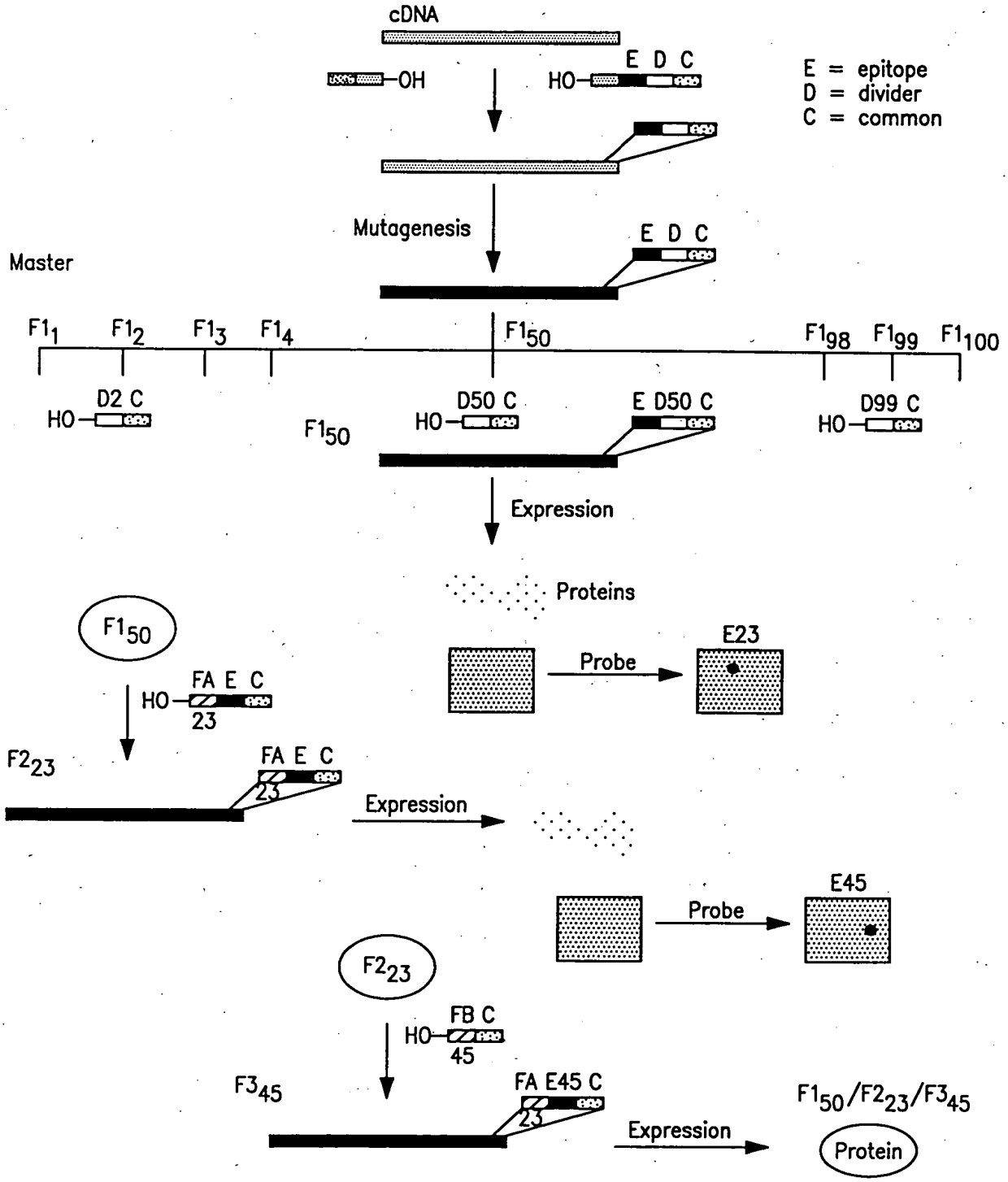
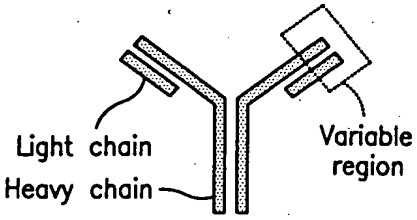
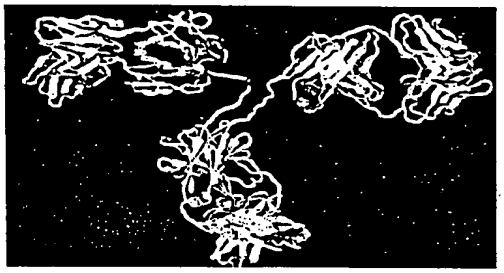


FIG. 4

Making a recombinant antibody library





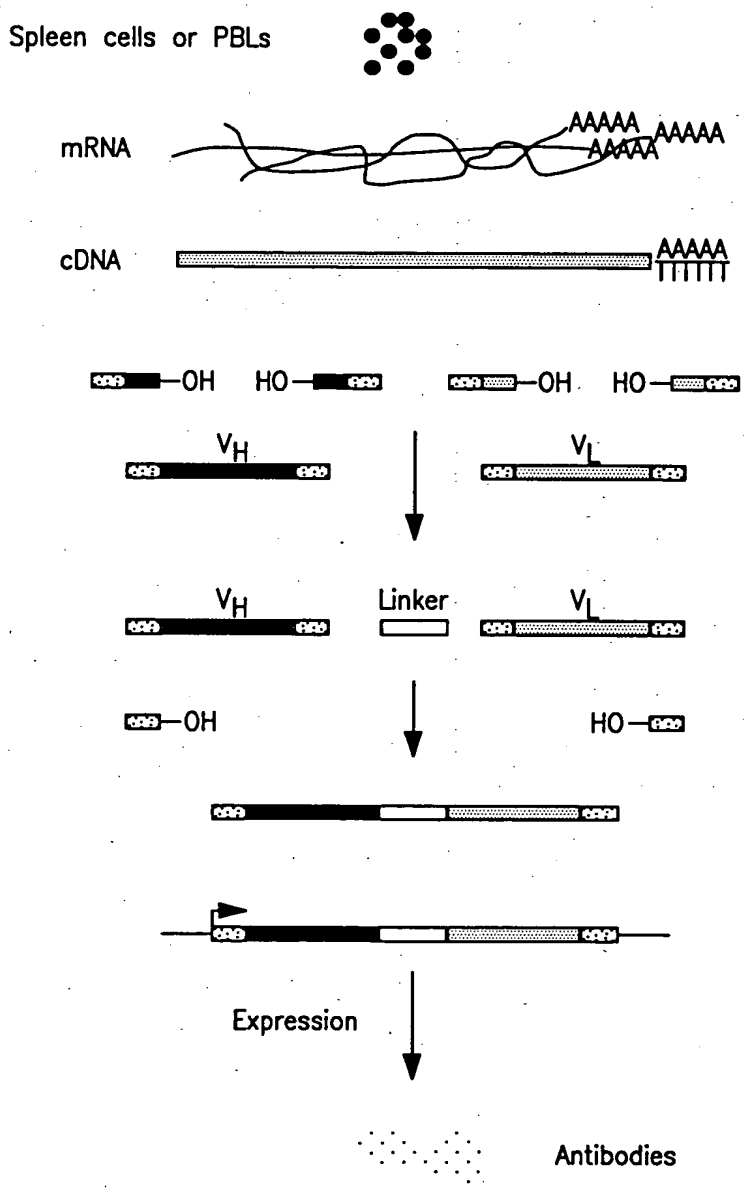


FIG. 5

Searching a recombinant antibody library

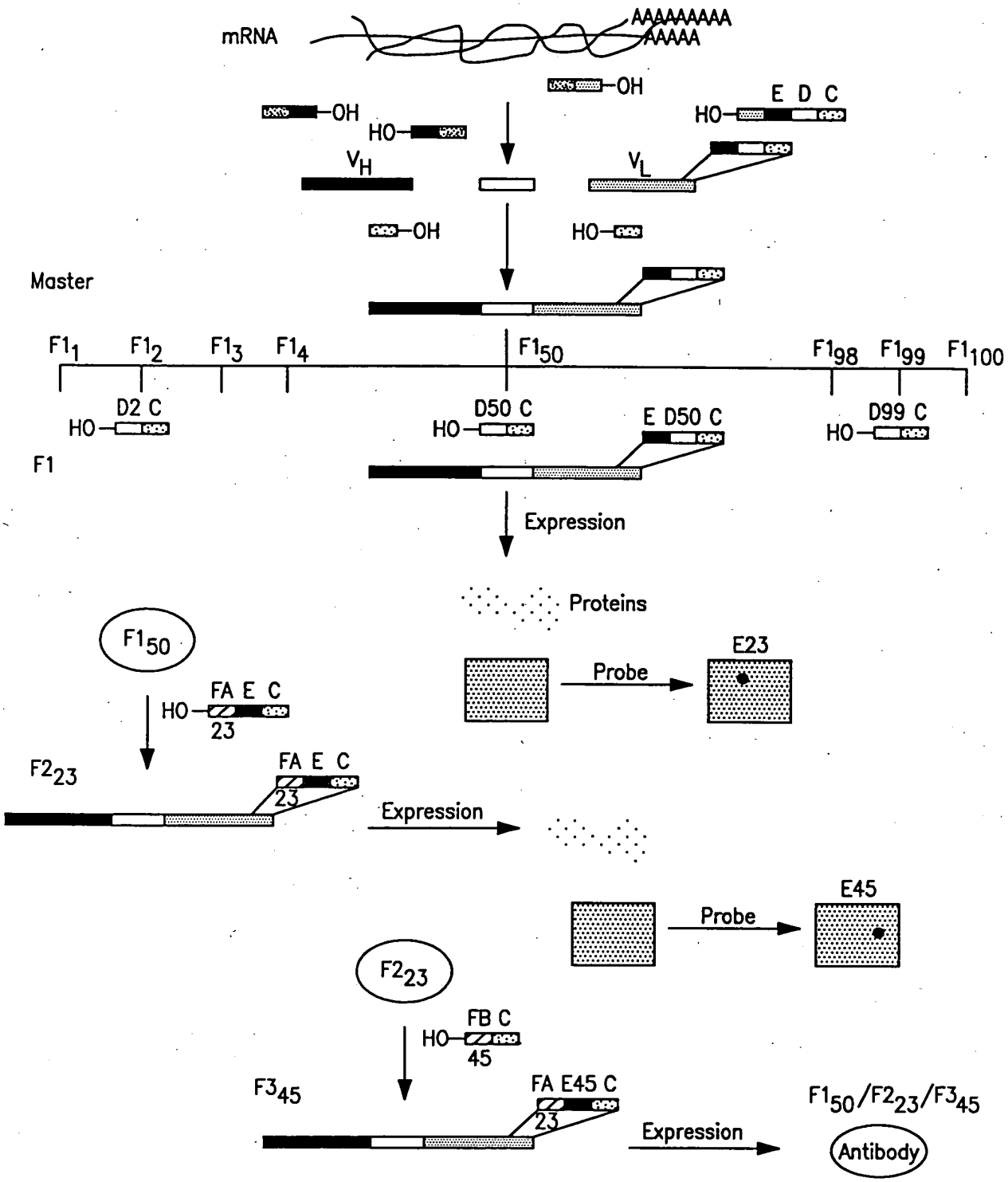
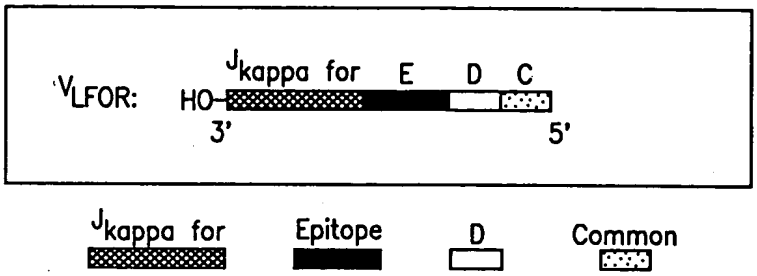
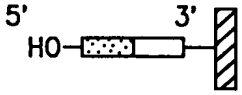


FIG. 8

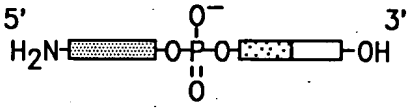
Making the V_{LFOR} primers: Solid phase synthesis



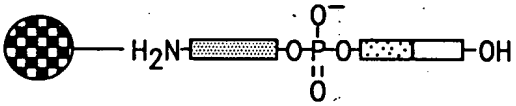
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA patch and ligating

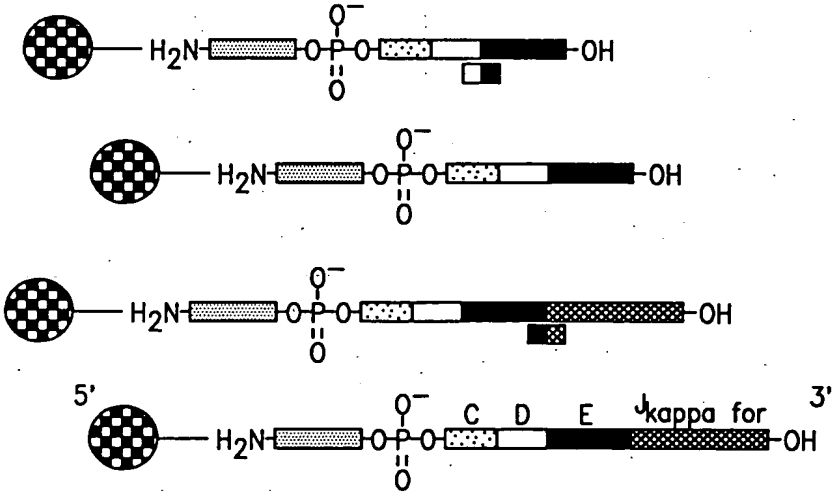


FIG. 10

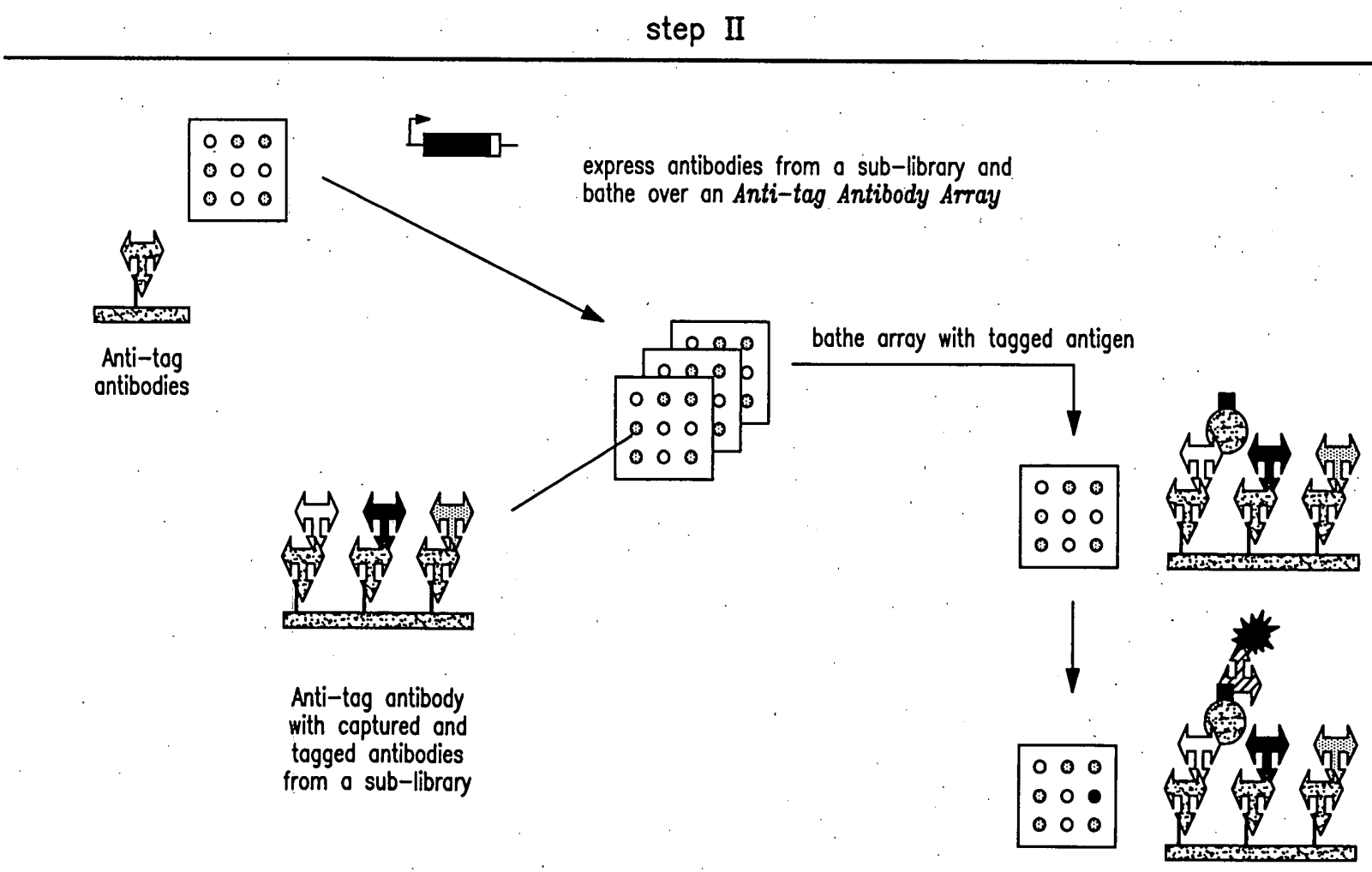
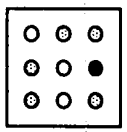

step II
express antibodies from a sub-library and bathe over an *Anti-tag Antibody Array*
Anti-tag antibodies
bathe array with tagged antigen
Anti-tag antibody with captured and tagged antibodies from a sub-library
ID spot containing the antigen with a labeled developing Ab

FIG. 14B

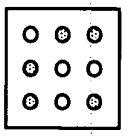
step III



Amplifiy the antibody genes from the identified sub-library using tag-specific PCR primers

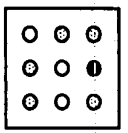
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

Express and purify the antibodies



Re-distribute over an ***Anti-tag Antibody Array***

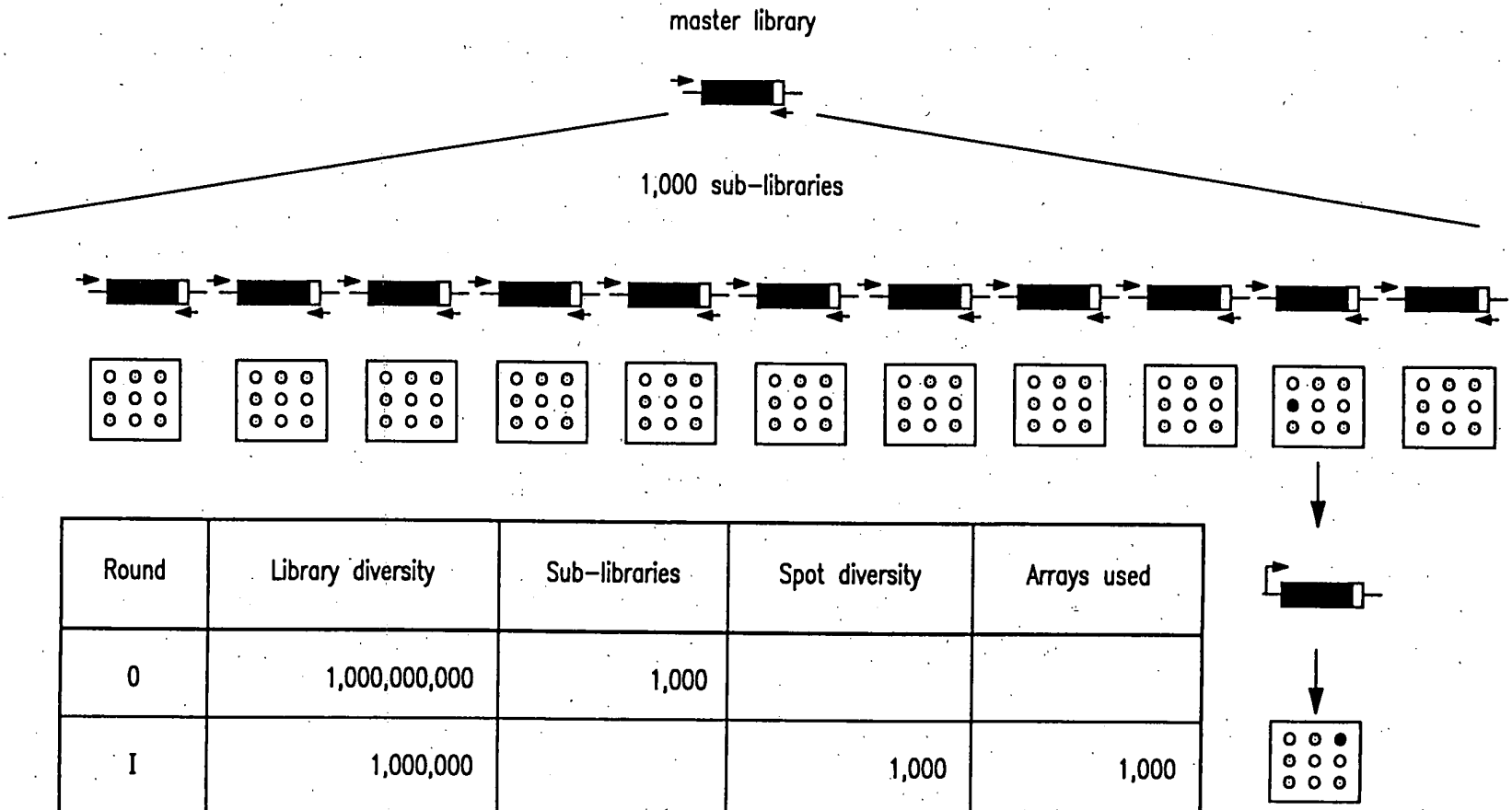
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb



Re-survey to ID the antibody of interest

FIG. 14C

summary



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIG. 14D

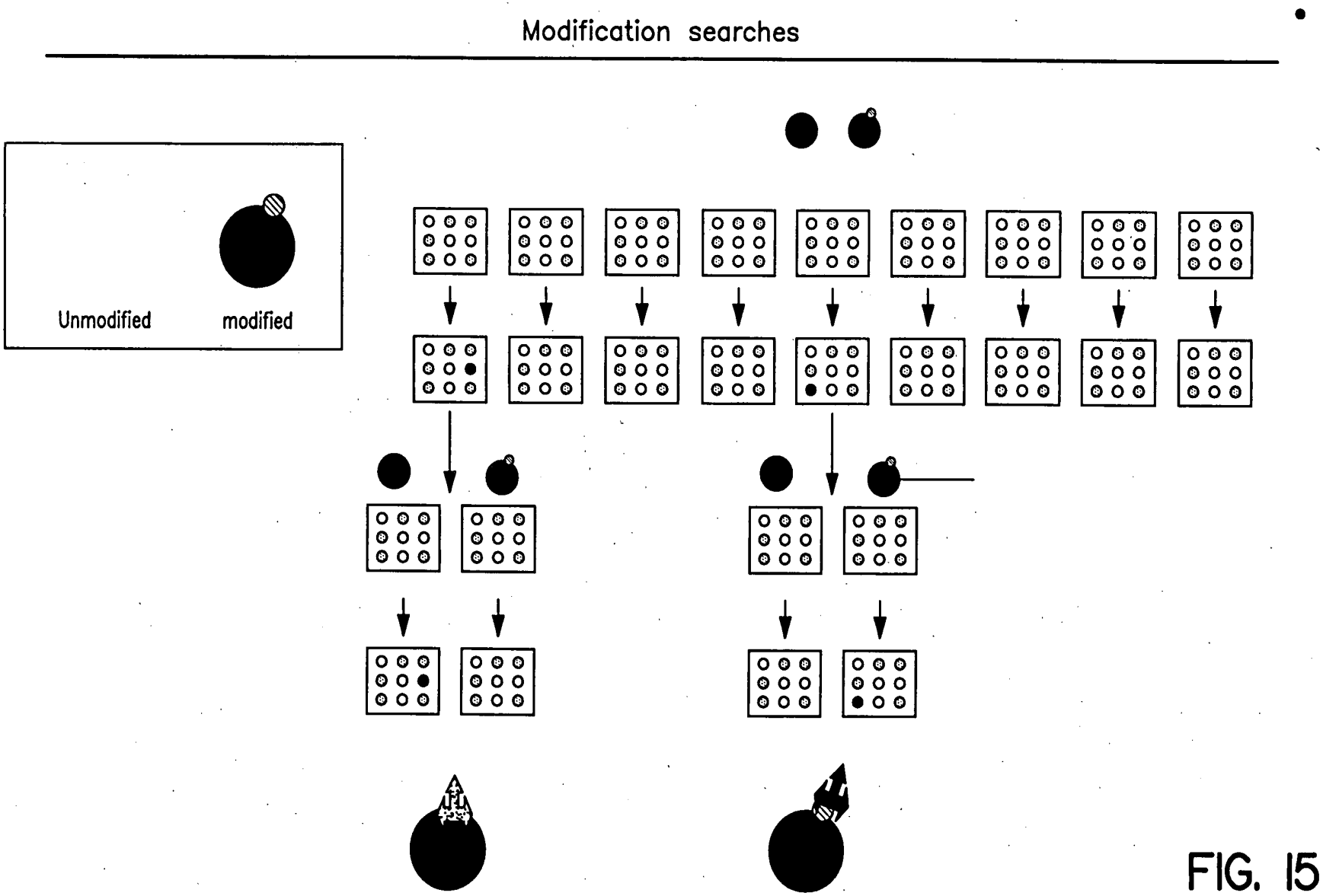


FIG. 15

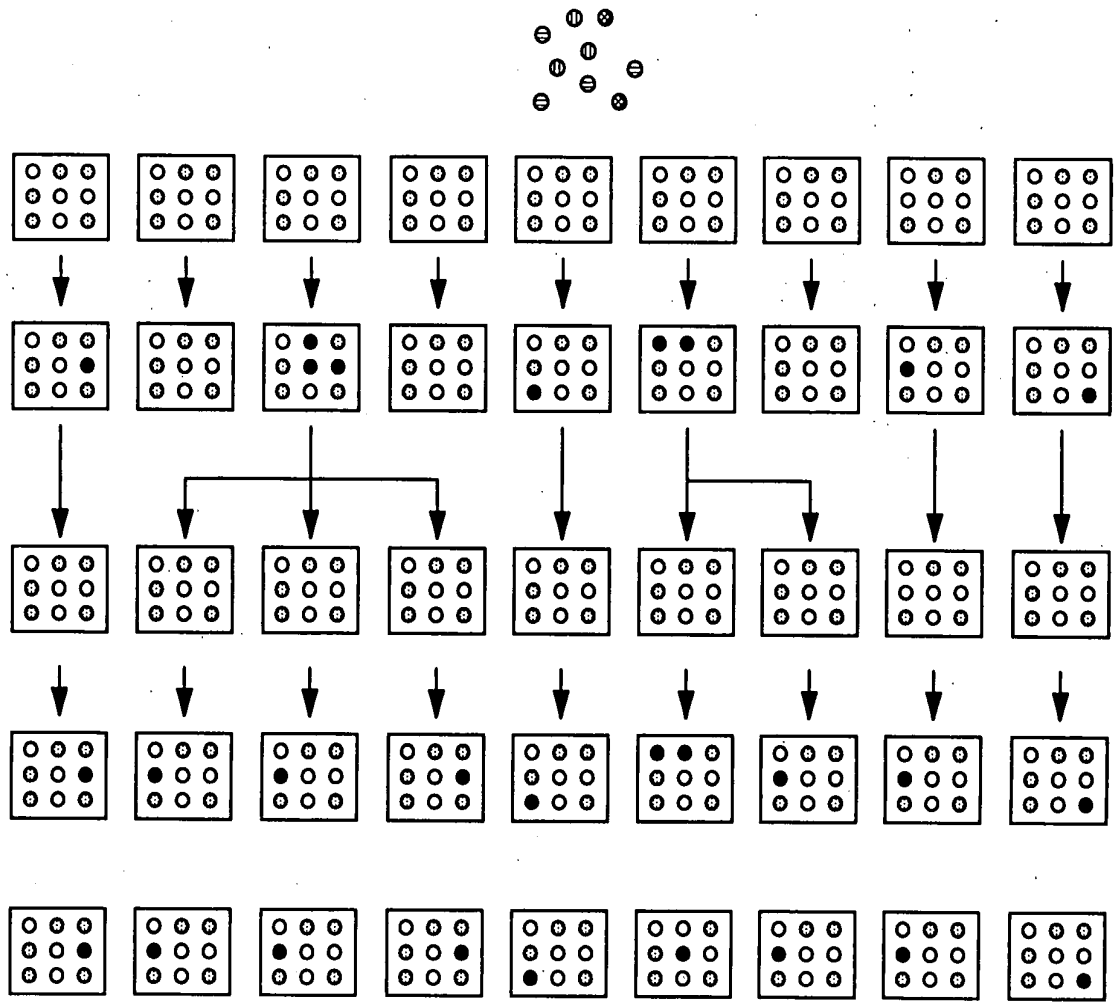
Simultaneous searches

Round Arrays Bait Probe

I 1,000 Abs Ags

II 1,000 Abs Ags

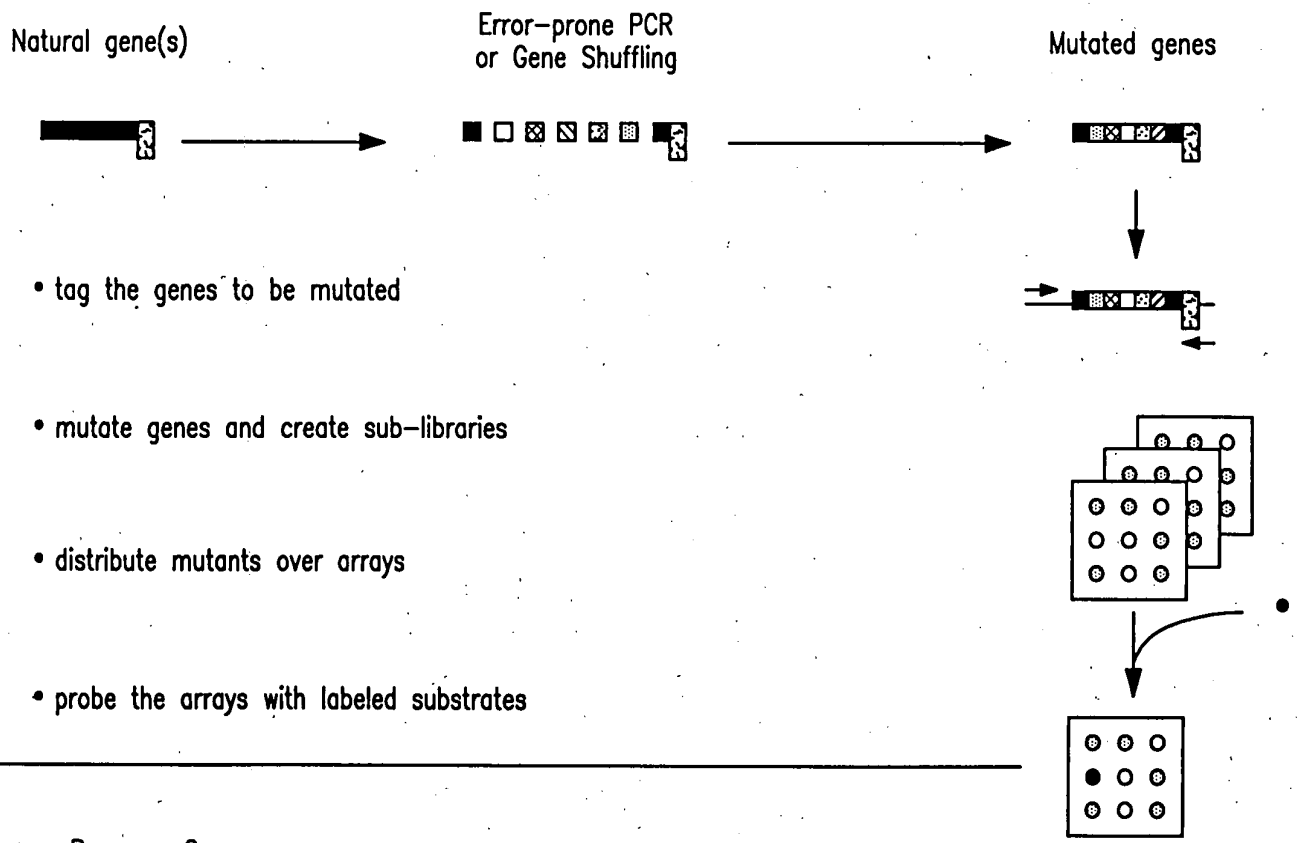
III 1,000 Ags Abs / 3,000

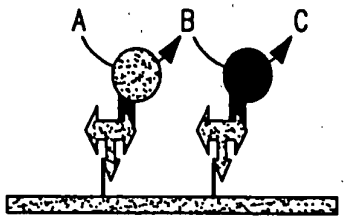


3 Arrays per Ag

FIG. 16

Protein interaction mapping





Spots can contain mixtures of enzymes
for detection or pathway engineering

FIG. 17

Protein interaction mapping

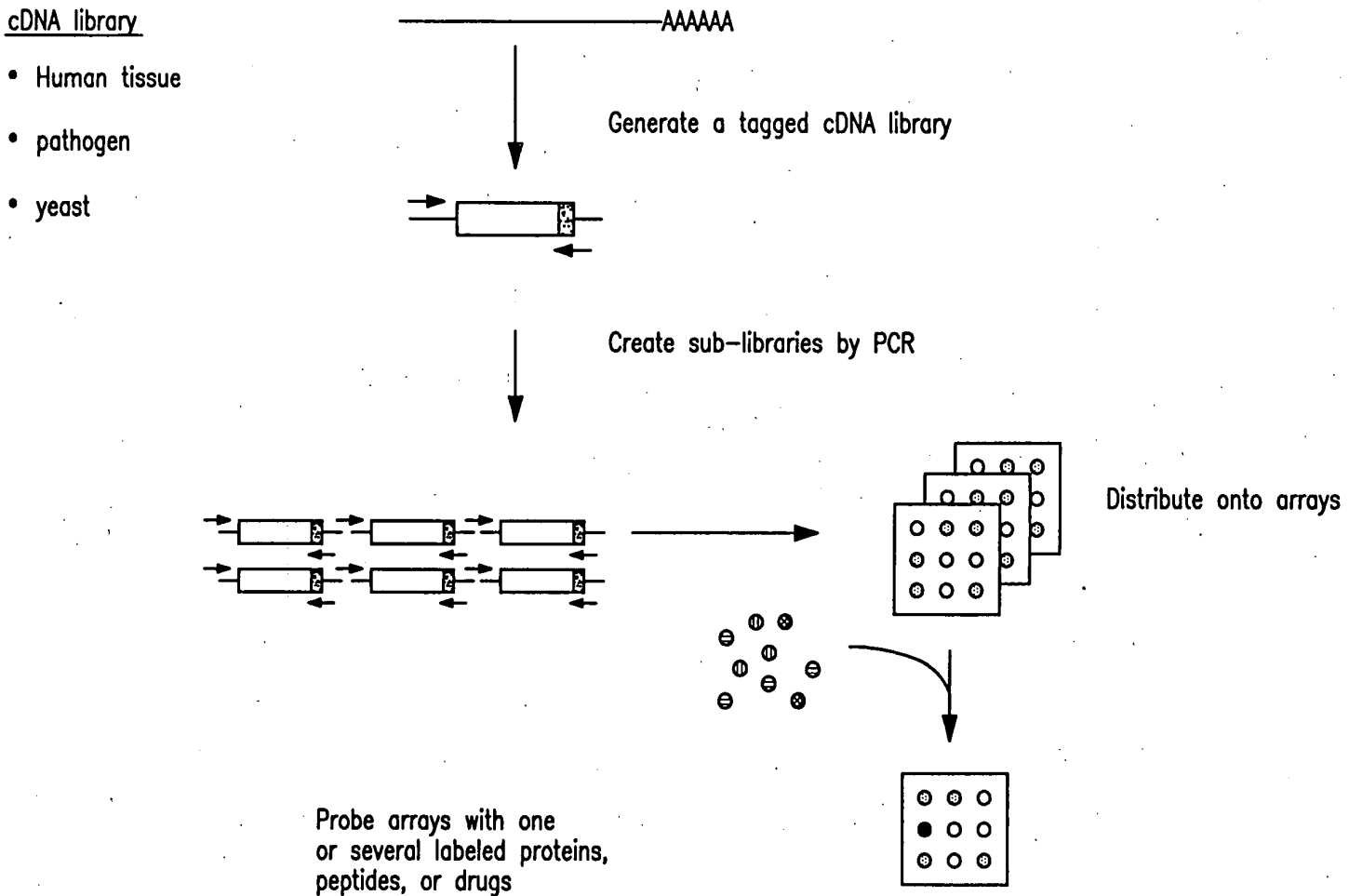


FIG. 18